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(57) Abstract

HLWAR77 polypeptides and polynucleotides and methods of producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods of utilizing HLWAR77 polypeptides and polynucleotides in the design of protocols for the treatment of infections and diseases.

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7TM RECEPTOR HLWAR77

This application is continuation-in-part application of U.S. Serial No 09/006,140 filed 13-Jan-98 which is herein incorporated by reference in its entirety. US Serial No 09/006,140 in turn claims the benefit of U.S. Provisional Application Nos. 60/049,332, filed June 11, 1997, and 60/067,253, filed December 2, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to G-protein coupled receptor family, hereinafter referred to as HLWAR77. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, Nature, 1991, 351:353-354). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., et al., Proc. Natl Acad. Sci., USA, 1987, 84:46-50; Kobilka, B.K., et al., Science, 1987, 238:650-656; Bunzow, J.R., et al., Nature, 1988, 336:783-787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., Science, 1991, 252:802-8).

For example, in one form of signal transduction, the effect of hormone binding is activation of the enzyme, adenylate cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide, GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein was shown to exchange GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to

represent transmembrane α-helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors (otherwise known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include, but are not limited to, calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorant, and cytomegalovirus receptors.

Most G-protein coupled receptors have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structure. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β-adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc. Rev., 1989, 10:317-331) Different G-protein α-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regulation of G-

protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

Over the past 15 years, nearly 350 therapeutic agents targeting 7 transmembrane (7 TM) receptors have been successfully introduced onto the market.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy

complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to HLWAR77 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such HLWAR77 polypeptides and polynucleotides. Such uses include the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy complex; pallido-ponto-nigral

degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others.

In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with HLWAR77 imbalance with the identified compounds. In particular, the preferred method for identifying agonist or antagonist of a HLWAR77 receptor of the present invention comprises:

contacting a cell expressing on the surface thereof the receptor, said receptor being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said receptor, with a compound to be screened under conditions to permit binding to the receptor; and

determining whether the compound binds to and activates or inhibits the receptor by measuring the level of a signal generated from the interaction of the compound with the receptor.

In a further preferred embodiment, the method further comprises conducting the identification of agonist or antagonist in the presence of labeled or unlabeled, A-18-F-NH_2 or F-8-F-NH_2.

In another embodiment of the method for identifying agonist or antagonist of a HLWAR77 receptor of the present invention comprises:

determining the inhibition of binding of a ligand to cells which have the receptor on the surface thereof, or to cell membranes containing the recetpor, in the presence of a candidate compound under conditions to permit binding to the receptor, and determining the amount of ligand bound to the receptor, such that a compound capable of causing reduction of binding of a ligand is an agonist or antagonist. Preferably the ligand is A-18-F-NH_2 or F-8-F-NH_2. Yet more preferably A-18-F-NH_2 or F-8-F-NH_2 is labeled.

Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HLWAR77 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"HLWAR77" or "HLWAR77 receptor" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said HLWAR77 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said HLWAR77.

"HLWAR77 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either

by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many

regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1):* 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol. 215:* 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al., J. Mol. Biol. 215*: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one

amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity(divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

The peptide defined herein by the notation "A-18-F-NH2" refers to a peptide with amino acid seqeunce H-Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Phe-NH2 (SEQ ID NO:7). Further, the pepeitde defined by the notation "F-8-F-NH2" refers to a peptide with amino acid seqeunce H-Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH2. Both peptides are described in Yang HY (SEQ ID NO:8), Fratta W, Majane EA, Costa E *Isolation, sequencing, synthesis, and pharmacological characterization of two brain neuropeptides that modulate the action of morphine*. Proc Natl Acad Sci U S A 1985 Nov;82(22):7757-61.

Polypeptides of the Invention

In one aspect, the present invention relates to HLWAR77 polypeptides (or HLWAR77 proteins). The HLWAR77 polypeptides include the polypeptides of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within HLWAR77 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at

least 97-99% are highly preferred. Preferably HLWAR77 polypeptides exhibit at least one biological activity of the receptor.

The HLWAR77 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the HLWAR77 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned HLWAR77 polypeptides. As with HLWAR77 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of HLWAR77 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of HLWAR77 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical

such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The HLWAR77 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to HLWAR77 polynucleotides. HLWAR77 polynucleotides include isolated polynucleotides which encode the HLWAR77 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, HLWAR77 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a HLWAR77 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. HLWAR77 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the HLWAR77 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under HLWAR77 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such HLWAR77 polynucleotides.

HLWAR77 of the invention is structurally related to other proteins of the G-protein coupled receptor family, as shown by the results of sequencing the cDNA encoding human HLWAR77. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 348 to 1610) encoding a polypeptide of 421 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 33% identity (using FASTA) in 300 amino acid residues with Neuropeptide Y receptor. Li X., Wu Y, North R.,Forte M.1992. J. Biol. Chem.,267, 9-12. The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 50% identity (using FASTA) in 730

nucleotide residues with Neuropeptide Y receptor. Li X, Wu Y, North R., Forthe M.1992. J. Biol. Chem. 267,9-122. Thus, HLWAR77 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

```
   1 CCATCCTAAT ACGACTCACT ATAGGGCTCG AGCGGCCGCC CGGGCAGGTT
  51 CTCGGCTCAC TGCAAGCTCC ACCTCCTGGG TTCACGCTAT TCTCCTGCCT
 101 CAGCCTCCTG AGTAGCTGGG ACTACAGGCG CCCGCCACCA CGCCTGGCTA
 151 ATTTTTTtGT ATTTTTAGTa GGGACGGCGT TTCACTGTGT TAGCCCAGAT
 201 GGTcTCCGTC TCCcGACCTc GtGATCCACC CACcTCGGCT TCCCAAAGTG
 251 CTGGGATTAC AGGCGTGAGC CACCGCGCCC GGCCAATTTC CTTTCTTAAT
 301 TGCCTctGCC CACCTCTTCT cTTCTGCTTC CATATTACAG GTTCATCATG
 351 AATGAGAAAT GGGACACAAA CTCTTCAGAA AACTGGCATC CCATCTGGAA
 401 TGTCAATGAC ACAAAGCATC ATCTGTACTC AGATATTAAT ATTACCTATG
 451 TGAACTACTA TcTTCACCAG CCTCAAGTGG CAGCAATCTT CATTATTTCC
 501 TACTTTCTGA TCTTCTTTTT GTGCATGATG GGAAATACTG TGGTTTGCTT
 551 TATTGTAATG AGGAACAAAC ATATGCACAC AGTCACTAAT CTCTTCATCT
 601 TAAACCTGGCCATAAGTGAT TTACTAGTTG GCATATTCTG CATGCCTATA
 651 ACACTGCTGG ACAATATTAT AGCAGGATGG CCATTTGGAA ACACGATGTG
 701 CAAGATCAGT GGATTGGTCC AGGGAATATC TGTCGCAGCT TCAGTCTTTA
 751 CGTTAGTTGC AATTGCTGTA GATAGGTTCC AGTGTGTGGT CTACCCTTTT
 801 AAACCAAAGC TCACTATCAA GACAGCGTTT GTCATTATTA TGATCATCTG
 851 GGTCCTAGCC ATCACCATTA TGTCTCCATC TGCAGTAATG TTACATGTGC
 901 AAGAAGAAAA ATATTACCGA GTGAGACTCA ACTCCCAGAA TAAAACCAGT
 951 CCAGTCTACT GGTGCCGGGA AGACTGGCCA AATCAGGAAA TGAGGAAGAT
1001 CTACACCACT GTGCTGTTTG CCAACATCTA CCTGGCTCCC CTCTCCCTCA
1051 TTGTCATCAT GTATGGAAGG ATTGGAATTT CACTCTTCAG GGCTGCAGTT
1101 CCTCACACAG GCAGGAAGAA CCAGGAGCAG TGGCACGTGG TGTCCAGGAA
1151 GAAGCAGAAG ATCATTAAGA TGCTCCTGAT TGTGGCCCTG CTTTTTATTC
1201 TCTCATGGCT GCCCCTGTGG ACTCTAATGA TGCTCTCAGA CTACGCTGAC
1251 CTTTCTCCAA ATGAACTGCA GATCATCAAC ATCTACATCT ACCCTTTTGC
1301 ACACTGGCTG GCATTCGGCA ACAGCAGTGT CAATCCCATC ATTTATGGTT
1351 TCTTCAACGA GAATTTCCGC CGTGGTTTCC AAGAAGCTTT CCAGCTCCAG
1401 CTCTGCCAAA AAAGAGCAAA GCCTATGGAA GCTTATGCCC TAAAAGCTAA
1451 AAGCCATGTG CTCATAAACA CATCTAATCA GCTTGTCCAG GAATCTACAT
1501 TTCAAAACCC TCATGGGGAA ACCTTGCTTT ATAGGAAAAG TGCTGAAAAA
1551 CCCCAACAGG AATTAGTGAT GGAAGAATTA AAAGAAACTA CTAACAGCAG
1601 TGAGATTTAA AAAGAGCTAG TGTGATAATC CTAACTCTAC TACGCATTAT
1651 ATATTTAAAT CCATTGCTTT TTGTGGCTTT GCACTTCAAA TTTTTCAAAG
1701 AATGTTCTAA ATAAAACATT TACTGAAAGC CCTCTCTGGC AAAAAAATTA
1751 AAAATAAACA AAAATGGTCA TAAGATCATA AACAATCTTA TGTTGTATAA
1801 AAATACGTAG AGTGACTTAG ACATGTTTGC ATGAATAAAT ATATTTCTAG
1851 AGAACAGTTA AAAAAAAAAA AAAAAAAAAA AAAAAAAA
```

[a] A nucleotide sequence of a human HLWAR77 (SEQ ID NO: 1).

Table 2[b]

1 MNEKWDTNSS ENWHPIWNVN DTKHHLYSDI NITYVNYYLH QPQVAAIFII
51 SYFLIFFLCM MGNTVVCFIV MRNKHMHTVT NLFILNLAIS DLLVGIFCMP
101 ITLLDNIIAG WPFGNTMCKI SGLVQGISVA ASVFTLVAIA VDRFQCVVYP
151 FKPKLTIKTA FVIIMIIWVL AITIMSPSAV MLHVQEEKYY RVRLNSQNKT
201 SPVYWCREDW PNQEMRKIYT TVLFANIYLA PLSLIVIMYG RIGISLFRAA
251 VPHTGRKNQE QWHVVSRKKQ KIIKMLLIVA LLFILSWLPL WTLMMLSDYA
301 DLSPNELQII NIYIYPFAHW LAFGNSSVNP IIYGFFNENF RRGFQEAFQL
351 QLCQKRAKPM EAYALKAKSH VLINTSNQLV QESTFQNPHG ETLLYRKSAE
401 KPQQELVMEE LKETTNSSEI *

[b] An amino acid sequence of a human HLWAR77 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding HLWAR77 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of Human placenta using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al.*, *Nature*, (1992) *355*:632-634; Adams, M.D., *et al.*, *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding HLWAR77 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 348 to 1610 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of HLWAR77 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding HLWAR77 variants comprising the amino acid sequence of HLWAR77 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3[c]

```
   1 AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT TTTGACCTC
  51 ATAGAAGACA CCGGGACCGA TCCAGCCTCC GGACTCTAGC CTAGGCCGC
 101 GGACGGATAA CAATTTCACA CAGGAAACAG CTATGACCAC TAGGCTTTT
 151 CAAAAAGCTA TTTAGGTGAC ACTATAGAAG GTACGCCTGC AGGTACCGG
 201 CCGGAATTCC CGGGTCGACC CACGCGTCCG CATAAGTGAT TTACTAGTT
 251 GCATATTCTG CATGCCTATA ACACTGCTGG ACAATATTAT AGCAGGATG
 301 CCATTTGGAA ACACGATGTG CAAGATCAGT GGATTGGTCC AGGGAATAT
 351 TGTCGCAGCT TCAGTCTTTA CGTTAGTTGC AATTGCTGTA GATAGGTTC
 401 AGTGTGTGGT CTACCCTTTT AAACCAAAGC TCACTATCAA GACAGCGTT
 451 GTCATTATTA TGATCATCTG GGTCCTAGCC ATCACCATTA TGTCTCCAT
 501 TGCAGTAATG TTACATGTGC AAGAAGAAAA ATATTACCGA GTGAGACTC
 551 ACTCCCAGAA TAAAACCAGT CCAGTCTACT GGTGCCGGGA AGACTGGCC
 601 AATCAGGAAA TGAGGAAGAT CTACACCACT GTGCTGTTTG CCAACATCT
 651 CCTGGCTCCC CTCTCCCTCA TTGTCATCAT GTATGGAAGG ATTGGAATT
 701 CACTCTTCAG GGCTGCAGTT CCTCACACAG GCAGGAAGAA CCAGGAGCA
 751 TGGCACGTGG TGTCCAGGAA GAAGCAGAAG ATCATTAAGA TGCTCCTGA
 801 TGTGGCCCTG CTTTTTATTC TCTCATGGCT GCCCCTGTGG ACTCTAATG
 851 TGCTCTCAGA CTACGCTGAC CTTTCTCCAA ATGAACTGCA GATCATCAA
 901 ATCTACATCT ACCCTTTTGC ACACTGGCTG GCATTCGGCA ACAGCAGTG
 951 CAATCCCATC ATTTATGGTT TCTTCAACGA GAATTTCCGC CGTGGTTTC
1001 AAGAAGCTTT CCAGCTCCAG CTCTGCCAAA AAAGAGCAAA GCCTATGGA
1051 GCTTATGCCC TAAAAGCTAA AAGCCATGTG CTCATAAACA CATCTAATC
1101 GCTTGTCCAG GAATCTACAT TTCAAAACCC TCATGGGGAA ACCTTGCTT
1151 ATAGGAAAAG TGCTGAAAAA CCCCAACAGG AATTAGTGAT GGAAGAATT
1201 AAAGAAACTA CTAACAGCAG TGAGATTTAA AAAGAGCTAG TGTGATAAT
1251 CTAACTCTAC TACGCATTAT ATATTTAAAT CCATTGCTTT TTGTGGCTT
1301 GCACTTCAAA TTTTTCAAAG AATGTTCTAA ATAAAACATT TACTGAAAG
1351 CCTCTCTGGC AAAAAAATTA AAAATAAACA AAAATGGTCA TAAGATCAT
1401 AACAATCTTA TGTTGTATAA AAATACGTAG AGTGACTTAG ACATGTTTG
1451 ATGAATAAAT ATATTTCTAG AGAACAGTTA AAAAAAAAAA AAAAAAAAA
1501 AAAAAAAA
```

[c] A partial nucleotide sequence of a human HLWAR77 (SEQ ID NO: 3).

Table 4[d]

1	PRVRISDLLV GIFCMPITLL DNIIAGWPFG NTMCKISGLV QGISVAASVF
51	TLVAIAVDRF QCVVYPFKPK LTIKTAFVII MIIWVLAITI MSPSAVMLHV
101	QEEKYYRVRL NSQNKTSPVY WCREDWPNQE MRKIYTTVLF ANIYLAPLSL
151	IVIMYGRIGI SLFRAAVPHT GRKNQEQWHV VSRKKQKIIK MLLIVALLFI
201	LSWLPLWTLM MLSDYADLSP NELQIINIYI YPFAHWLAFG NSSVNPIIYG
251	FFNENFRRGF QEAFQLQLCQ KRAKPMEAYA LKAKSHVLIN TSNQLVQEST
301	FQNPHGETLL YRKSAEKPQQ ELVMEELKET TNSSEI*

[d] A partial amino acid sequence of a human HLWAR77 (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding HLWAR77 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the HLWAR77 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding HLWAR77 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x

Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine

techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the HLWAR77 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If HLWAR77 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

HLWAR77 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of HLWAR77 polynucleotides for use as diagnostic reagents. Detection of a mutated form of HLWAR77 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of HLWAR77. Individuals carrying mutations in the HLWAR77 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HLWAR77 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be

detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising HLWAR77 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to

narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, through detection of mutation in the HLWAR77 gene by the methods described.In addition, bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of HLWAR77 polypeptide or HLWAR77 mRNA.

Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an HLWAR77, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-

parkinsonism-amyotrophy complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrom, which comprises:

(a) a HLWAR77 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ;

(b) a nucleotide sequence complementary to that of (a);

(c) a HLWAR77 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or

(d) an antibody to a HLWAR77 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the HLWAR77 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the HLWAR77 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman,

using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against HLWAR77 polypeptides may also be employed to treat infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer

pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with HLWAR77 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to

narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering HLWAR77 polypeptide via a vector directing expression of HLWAR77 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a HLWAR77 polypeptide wherein the composition comprises a HLWAR77 polypeptide or HLWAR77 gene. The vaccine formulation may further comprise a suitable carrier. Since HLWAR77 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The HLWAR77 polypeptide of the present invention may be employed in a process for screening for compounds which bind to and activate the HLWAR77 polypeptides of the present invention (called agonists), or inhibit the interaction of the HLWAR77 polypeptides with receptor ligands (called antagonists).

Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be

structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

HLWAR77 proteins are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate HLWAR77 on the one hand and which can inhibit the function of HLWAR77 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; cancers; diabetes; asthma; Parkinson's disease; both acute and congestive heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; chronic renal failure; renal disease; impaired glucose tolerance; seizure disorder; depression; anxiety; obsessive compulsive disorder; affective neurosis/disorder; depressive neurosis/disorder; anxiety neurosis; dysthymic disorder; behavior disorder; mood disorder; shizophrenia; psychosexual dysfunction; sex disorder; sexual disorder; disturbed biological and circadian rhythms; feeding disorders, such as anorexia, bulimia, cachexia, and obesity; Cushing's syndrome / disease; basophil adenoma; prolactinoma; hyperprolactinemia; hypopituitarism; hypophysis tumor / adenoma; hypothalamic diseases; Froehlich's syndrome; adenohypophysis disease; hypophysis disease; hypophysis tumor / adenoma; pituitary growth hormone; adenohypophysis hypofunction; adrenohpophysis hyperfunction; hypothalamic hypogonadism; Kallman's syndrome (anosmia, hyposmia); functional or psychogenic amenorrhea; hypopituitarism; hypothalamic hypothyroidism; hypothalamic-adrenal dysfunction; idiopathic hyperprolactinemia; hypothalamic disorders of growth hormone deficiency; idiopathic growth hormone deficiency; dwarfism; gigantism; acromegaly; disturbed biological and circadian rhythms; and sleep disturbances associated with such diseases as neurological disorders, heart and lung diseases, mental illness, and addictions; migraine; hyperalgesia; enhanced or exaggerated sensitivity to pain, such as hyperalgesia, causalgia and allodynia; acute pain; burn pain; atypical facial pain; neuropathic pain; back pain; complex regional pain syndromes I and II; arthritic pain; sports injury pain; pain related to infection, e.g., HIV, post-polio syndrome, and post-herpetic neuralgia; phantom limb pain; labour pain; cancer pain; post-chemotherapy pain; post-stroke pain; post-operative pain; neuralgia; and tolerance to narcotics or withdrawal from narcotics; sleep disorders; sleep apnea; narcolepsy; insomnia; parasomnia; jet-lag syndrome; and other neurodegenerative disorders, which includes nosological entities such as disinhibition-dementia-parkinsonism-amyotrophy complex; pallido-ponto-nigral degeneration; and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome.

In general, such screening procedures involve providing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, Drosophila or E. coli. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express the HLWAR77 polypeptide. The expressed receptor is then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

One such screening procedure involves the use of melanophores which are transfected to express the HLWAR77 polypeptide of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed to screen for a compound which inhibits activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both the receptor ligand, such as A-18-F-NH_2 or F-8-F-NH_2, and a compound to be screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, i.e., inhibits activation of the receptor.

The technique may also be employed for screening of compounds which activate the receptor by contacting such cells with compounds to be screened and determining whether such compound generates a signal, i.e., activates the receptor.

Other screening techniques include the use of cells which express the HLWAR77 polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction or pH changes, is then measured to determine whether the potential compound activates or inhibits the receptor.

Another screening technique involves expressing the HLWAR77 polypeptide in which the receptor is linked to phospholipase C or D. Representative examples of such cells include, but are not limited to, endothelial cells, smooth muscle cells, and embryonic kidney cells. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase second signal.

Another method involves screening for compounds which are antagonists, and thus inhibit activation of the receptor polypeptide of the present invention by determining inhibition of binding of labeled ligand, such as A-18-F-NH_2 or F-8-F-NH_2, to cells which have the receptor on the surface thereof, or cell membranes containing the receptor. Such a method involves transfecting a eukaryotic cell with DNA encoding the HLWAR77 polypeptide such that the cell expresses the receptor on its surface. The cell is then contacted with a potential antagonist in the presence of a

labeled form of a ligand, such as A-18-F-NH_2 or F-8-F-NH_2. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity associated with transfected cells or membrane from these cells. If the compound binds to the receptor, the binding of labeled ligand to the receptor is inhibited as determined by a reduction of labeled ligand which binds to the receptors. This method is called binding assay. Naturally this same technique can be used to look for an agonist.

Another screening procedure involves the use of mammalian cells (CHO, HEK 293, Xenopus Oocytes, RBL-2H3, etc) which are transfected to express the receptor of interest. The cells are loaded with an indicator dye that produces a fluorescent signal when bound to calcium, and the cells are contacted with a test substance and a receptor agonist, such as A-18-F-NH_2 or F-8-F-NH_2. Any change in fluorescent signal is measured over a defined period of time using, for example, a fluorescence spectrophotometer or a fluorescence imaging plate reader. A change in the fluorescence signal pattern generated by the ligand indicates that a compound is a potential antagonist or agonist for the receptor.

Another screening procedure involves use of mammalian cells (CHO, HEK293, Xenopus Oocytes, RBL-2H3, etc.) which are transfected to express the receptor of interest, and which are also transfected with a reporter gene construct that is coupled to activation of the receptor (for example, luciferase or beta-galactosidase behind an appropriate promoter). The cells are contacted with a test substance and the receptor agonist (ligand), such as A-18-F-NH_2 or F-8-F-NH_2, and the signal produced by the reporter gene is measured after a defined period of time. The signal can be measured using a luminometer, spectrophotometer, fluorimeter, or other such instrument appropriate for the specific reporter construct used. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor.

Another screening technique for antagonists or agonits involves introducing RNA encoding the HLWAR77 polypeptide into Xenopus oocytes (or CHO, HEK 293, RBL-2H3, etc.) to transiently or stably express the receptor. The receptor oocytes are then contacted with the receptor ligand, such as A-18-F-NH_2 or F-8-F-NH_2, and a compound to be screened. Inhibition or activation of the receptor is then determined by detection of a signal, such as, cAMP, calcium, proton, or other ions.

Another method involves screening for HLWAR77 polypeptide inhibitors by determining inhibition or stimulation of HLWAR77 polypeptide-mediated cAMP and/or adenylate cyclase accumulation or dimunition. Such a method involves transiently or stably transfecting a eukaryotic cell with HLWAR77 polypeptide receptor to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the presence of HLWAR77 polypeptide ligand,

such as A-18-F-NH_2 or F-8-F-NH_2. The changes in levels of cAMP is then measured over a defined period of time, for example, by radio-immuno or protein binding assays (for example using Flashplates or a scintillation proximity assay). Changes in cAMP levels can also be determined by directly measuring the activity of the enzyme, adenylyl cyclase, in broken cell preparations. If the potential antagonist binds the receptor, and thus inhibits HLWAR77 polypeptide-ligand binding, the levels of HLWAR77 polypeptide-mediated cAMP, or adenylate cyclase activity, will be reduced or increased.

Another screening method for agonists and antagonists relies on the endogenous pheromone response pathway in the yeast, Saccharomyces cerevisiae. Heterothallic strains of yeast can exist in two mitotically stable haploid mating types, MATa and MATa. Each cell type secretes a small peptide hormone that binds to a G-protein coupled receptor on opposite mating-type cells which triggers a MAP kinase cascade leading to G1 arrest as a prelude to cell fusion. Genetic alteration of certain genes in the pheromone response pathway can alter the normal response to pheromone, and heterologous expression and coupling of human G-protein coupled receptors and humanized G-protein subunits in yeast cells devoid of endogenous pheromone receptors can be linked to downstream signaling pathways and reporter genes (e.g., U.S. Patents 5,063,154; 5,482,835; 5,691,188). Such genetic alterations include, but are not limited to, (i) deletion of the STE2 or STE3 gene encoding the endogenous G-protein coupled pheromone receptors; (ii) deletion of the FAR1 gene encoding a protein that normally associates with cyclin-dependent kinases leading to cell cycle arrest; and (iii) construction of reporter genes fused to the FUS1 gene promoter (where FUS1 encodes a membrane-anchored glycoprotein required for cell fusion). Downstream reporter genes can permit either a positive growth selection (e.g., histidine prototrophy using the FUS1-HIS3 reporter), or a colorimetric, fluorimetric or spectrophotometric readout, depending on the specific reporter construct used (e.g., b-galactosidase induction using a FUS1-LacZ reporter).

The yeast cells can be further engineered to express and secrete small peptides from random peptide libraries, some of which can permit autocrine activation of heterologously expressed human (or mammalian) G-protein coupled receptors (Broach, J.R. and Thorner, J. Nature 384: 14-16, 1996; Manfredi et al., Mol. Cell. Biol. 16: 4700-4709, 1996). This provides a rapid direct growth selection (e.g, using the FUS1-HIS3 reporter) for surrogate peptide agonists that activate characterized or orphan receptors. Alternatively, yeast cells that functionally express human (or mammalian) G-protein coupled receptors linked to a reporter gene readout (e.g., FUS1-LacZ) can be used as a platform for high-throughput screening of known ligands, fractions of biological extracts and libraries of chemical compounds for either natural or surrogate ligands.

Functional agonists of sufficient potency (whether natural or surrogate) can be used as screening tools in yeast cell-based assays for identifying G-protein coupled receptor antagonists. For example, agonists will promote growth of a cell with *FUS-HIS3* reporter or give positive readout for a cell with *FUS1-LacZ*. However, a candidate compound which inhibits growth or negates the positive readout induced by an agonist is an antagonist. For this purpose, the yeast system offers advantages over mammalian expression systems due to its ease of utility and null receptor background (lack of endogenous G-protein coupled receptors) which often interferes with the ability to identify agonists or antagonists.

The present invention also provides a method for identifying new ligands not known to be capable of binding to an HLWAR77 polypeptides. The screening assays described above for identifying agonists may be used to identify new ligands.

The present invention also contemplates agonists and antagonists obtainable from the above described screening methods.

Examples of potential HLWAR77 polypeptide receptor antagonists include peptidomimetics, synthetic organic molecules, natural products, antibodies, etc. which bind to the receptor but do not elicit a second messenger response such that the activity of the receptor is prevented.

Potential antagonists also include proteins which are closely related to the ligand of the HLWAR77 polypeptide receptor, i.e. a fragment of the ligand, which have lost biological function, and when they bind to the HLWAR77 polypeptide receptor, elicit no response.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, and ligands for HLWAR77 polypeptides, which comprises:
(a) a HLWAR77 polypeptide, preferably that of SEQ ID NO:2; and further preferably comprises labeled or unlabeled A-18-F-NH_2 or F-8-F-NH_2;
(b) a recombinant cell expressing a HLWAR77 polypeptide, preferably that of SEQ ID NO:2; and further preferably comprises labeled or unlabeled A-18-F-NH_2 or F-8-F-NH_2; or
(c) a cell membrane expressing HLWAR77 polypeptide; preferably that of SEQ ID NO: 2; and further preferably comprises labeled or unlabled A-18-F-NH_2 or F-8-F-NH_2.

It will be appreciated that in any such kit, (a), (b), or (c) may comprise a substantial component.

As noted above, a potential antagonist is a small molecule which binds to the HLWAR77 polypeptide receptor, making it inaccessible to ligands such that normal biological activity is prevented. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules.

Potential antagonists also include soluble forms of HLWAR77 polypeptide receptor, e.g., fragments of the receptor, which bind to the ligand and prevent the ligand from interacting with membrane bound HLWAR77 polypeptide receptors.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of HLWAR77 activity.

If the activity of HLWAR77 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the HLWAR77, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of HLWAR77 polypeptides still capable of binding the ligand in competition with endogenous HLWAR77 may be administered. Typical embodiments of such competitors comprise fragments of the HLWAR77 polypeptide.

In still another approach, expression of the gene encoding endogenous HLWAR77 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979) 6:3073; Cooney *et al., Science* (1988) 241:456; Dervan *et al., Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of HLWAR77 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates HLWAR77, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of HLWAR77 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and

expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of HLWAR77 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a

polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1: Mammalian Cell Expression

The receptors of the present invention are expressed in either human embryonic kidney 293 (HEK293) cells or adherent dhfr CHO cells. To maximize receptor expression, typically all 5' and 3' untranslated regions (UTRs) are removed from the receptor cDNA prior to insertion into a pCDN or pCDNA3 vector. The cells are transfected with individual receptor cDNAs by lipofectin and selected in the presence of 400 mg/ml G418. After 3 weeks of selection, individual clones are picked and expanded for further analysis. HEK293 or CHO cells transfected with the vector alone serve as negative controls. To isolate cell lines stably expressing the individual receptors, about 24 clones are typically selected and analyzed by Northern blot analysis. Receptor mRNAs are generally detectable in about 50% of the G418-resistant clones analyzed.

Example 2 Ligand bank for binding and functional assays.

A bank of over 200 putative receptor ligands has been assembled for screening. The bank comprises: transmitters, hormones and chemokines known to act via a human seven transmembrane (7TM) receptor; naturally occurring compounds which may be putative agonists for a human 7TM receptor, non-mammalian, biologically active peptides for which a mammalian counterpart has not yet been identified; and compounds not found in nature, but which activate 7TM receptors with unknown natural ligands. This bank is used to initially screen the receptor for known ligands, using both functional (i.e . calcium, cAMP, microphysiometer, oocyte electrophysiology, etc, see below) as well as binding assays.

Example 3: Ligand Binding Assays

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a receptor is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators, such as nucleotides, are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific receptor binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

Example 4: Functional Assay in Xenopus Oocytes

Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual Xenopus oocytes in response to agonist exposure. Recordings are made in Ca2+ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 5: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the activation of a receptor which is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled receptor of the present invention.

Example 6: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the 7TM receptor of the invention is also functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 7: Calcium and cAMP Functional Assays

7TM receptors which are expressed in HEK 293 cells have been shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were

observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day > 150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP flucuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

Example 8: Cloning of full length sequence

An EST homologous to the 7TM superfamily sequences was identified. Oligonucleotides from these regions were designed to obtain the 5' coding region using the Marathon PCR technology. The 5' oligos were (AAAGACTGAAGCTGCGACAGATATTC (SEQ ID NO:5) AND CCTGGACCAATCCACTGATCTTGC (SEQ ID NO:6)). Fragments of approximately 1200 bp (5' fragment) were obtained and subcloned into PCR 2.1 vector (Invitrogene) and sequenced. Sequence analysis showed an open reading frame starting with nucleotide 348(ATG) and ending at nucleotide 1610 (TGA). The coding region is 1263 nucleotides which encodes a protein of 421 amino acids. The protein is predicted to have a molecular weight of approximately 46,310.

Example 9:

We have discovered that A-18-F-NH_2 and F-8-F-NH_2 are ligands for HLWAR77 receptor by the following experiment. HEK-293 cells were co-transfected with a mammalian expression plasmid encoding HLWAR77, along with cDNAs encoding G 15 (Wilkie T.M. et al *Proc Natl Acad Sci USA* 1991 88:10049-10053) and a chimeric G-protein refered to as Gqi5 (Conklin BR et al., *Nature* 1993 363:274-276), and assayed on FLIPR for a calcium mobilization response following addition of as A-18-F-NH_2 or F-8-F-NH_2. A robust, dose dependent (EC50s ~ 3-5 nM), calcium mobilization response was detected following addition of either of these ligands to cells transfected with HLWAR77 and the G-proteins. The agonist peptides did not stimulate a calcium mobilization response in HEK-293 cells transfected only with HLWAR77, nor was a response detected to these ligands in HEK-293 cells transfected only with G 15 and Gqi5. The cDNAs for both the receptor and the G-proteins had to be expressed in the HEK-293 in order to detect a functional response to these agonists.

Subsequently it has been determined that HEK-293 cells co-transfected with HLWAR77, and a different chimeric G-protein, Gqo5 (Conklin BR et al *Nature* 1993 363:274-276), give a

greater calcium mobilization response than cells co-transfected with HLWAR77 and either G 15, Gqi5, or a mixture of the 2 cDNAs. Additional G-protein must be present HEK-293 cells in order to detect calcium signalling mediated through HLWAR77. Thus in the case of using HEK-293, as described above, additional G-protein(s) is (are) required to run screens for agonists and antagonists. It is possible that HLWAR77 expressed in another cell, for example RBL-2H3, may signal through calcium pathways without requiring additional G-protein, as has been noted for the C5a receptor (Martino, J.A. et al J Biol Chem. 1994 269:14446-14450), which in some cells also requires additional G-protein for functional coupling (Amatuda T.T. et al *J Biol Chem* 1993 268:10139-10144).

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the HLWAR77 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the HLWAR77 polypeptide of SEQ ID NO2.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a HLWAR77 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a HLWAR77 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a HLWAR77 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a HLWAR77 polypeptide.

10. A HLWAR77 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. A method for identifying agonist or antagonist of a polypeptide of claim 10 which comprises:

contacting a cell expressing on the surface thereof the polypeptide, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the polypeptide.

13. A method of claim 12 which further comprises conducting the identification of agonist or antagonist in the presence of labeled or unlabeled as A-18-F-NH_2 or F-8-F-NH_2.

14. A method for identifying agonist or antagonist of a polypeptide of claim 10 which comprises:

determining the inhibition of binding of a ligand to cells which have the polypeptide on the surface thereof, or to cell membranes containing the polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide, and determining the amount of ligand bound to the polypeptide, such that a compound capable of causing reduction of binding of a ligand is an agonist or antagonist.

15. A method of claim 14 in which a ligand is labeled or unlabeled as A-18-F-NH2 or F-8-F-NH2.

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27282

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 435/7.2, 69.1, 325; 530/350; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 69.1, 325; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, MEDLINE, CAPLUS, GENBANK, SWISSPROT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HODGSON J. Receptor Screening and the Search for New Pharmaceuticals. Bio/Technology. September 1992, Vol. 10, pages 973-977, especially page 975.	12-15
A	GETHER U. et al. G Protein-coupled Receptors. J. Biol. Chem. 17 July 1998, Vol. 273(29), pages 17979-17982, especially page 17981.	12-15
A	STRADER C.D. et al. Structure and Function of G Protein-Coupled Receptors. Annu. Rev. Biochem. 1994. Vol. 63, pages 101-132, whole reference.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 FEBRUARY 2000

Date of mailing of the international search report
09 MAY 2000

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27282

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07H 21/04; C07K 1/00, 14/00; C12N 5/00; C12P 21/06; G01N 33/53

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27282

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FRASER C.M. et al. Molecular properties and Regulation of G-Protein Coupled Receptors. 1994. Vol. 49, pages 113-156, whole reference.	1-15